STUDY OF THE STABILITY AND OXYGEN SOLUBILITY OF PERFLUOROCARBON EMULSIONS

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Abstract: The ageing mechanisms of perfluorocarbon emulsions were studied. Emulsions with the same concentration of the perfluorohexane, 50 % (w/v), and of three different surfactants, lecithin, Span 20 and Pluronic F-68, were prepared by sonication and their stability was evaluated through the analysis of the evolution of the mean micelle size. The results indicate that two different ageing mechanisms may take place depending on the emulsifier used. The emulsions prepared with lecithin and Span 20 loose stability by coalescence while Ostwald ripening is the primary coarsening mechanism of the Pluronic F-68 -emulsion.

The oxygen solubility in the perfluorocarbon emulsions was also studied using an enzymatic method. The results obtained show that the oxygen solubility in the three emulsions are of the same order of magnitude, thus indicating that the surfactant does not play a significant role in this property.

Keywords: perfluorocarbon emulsions, emulsions stability, molecular diffusion, Ostwald ripening, coalescence, oxygen solubility

INTRODUCTION

The use of perfluorocarbons (PFCs) as oxygen carriers were first proposed in 1966, when Clark and Gollan (1966) at the University of Cincinnati demonstrated the capacity of these liquids to support animal life through liquid breathing. PFCs are highly fluorinated, inert organic compounds that can dissolve large volumes of respiratory gases such as oxygen and carbon dioxide. Liquid PFCs are colorless, odorless and non-corrosive (Lowe, 1998). As they are immiscible in aqueous systems, including biological fluids like plasma, they have to be emulsified for intravascular administration. The transport and delivery of oxygen *in vivo* by other means than the red blood cells has become one of the most challenging research topics of the last 25 years. Perfluorocarbon emulsions are one of the main candidates for a safe and reliable blood substitute (Riess, 1992; Postel et al., 1994; Lowe, 1998).

Perfluorocarbon-based emulsions with application in the biomedical field have known some developments and drawbacks (Riess, 1992). Apart from suitable thermophysical properties and inertness, stability plays a primary role for their use as injectable oxygen carriers, contrast agents, drug delivery systems or cell culture media supplements (Riess, 1992).

Since emulsions are thermodynamically unstable systems, they do not form spontaneously and their thermodynamic fate is phase separation. There has been a considerable effort to formulate stable perfluorocarbon emulsions and to understand the driving forces that cause their ageing. Emulsion stability can be studied through the evolution of the micelles size, where the increase in size indicates the loss of stability. Two main mechanisms have been proposed by which oil-in-water emulsions loose stability: coalescence and Ostwald ripening or molecular diffusion.

AGEING MECHANISMS

Coalescence is the formation of a larger droplet from the merging of smaller ones. This requires that the small droplets come into contact, with the thinning and disruption of the liquid surfactant film that covers them. Emulsion degradation by coalescence is characterized by a broadening in particle size distribution, where the increase in mean micelle size accelerates with time, generally sudden and unpredictable. According to the Van den Tempel Theory (1953) coalescence occurs when the volume of the particle increases exponentially with time

$$\bar{a}^3 = \overline{a_0}^3 \exp(Kt) \qquad \textbf{(1)}$$

where $\overline{a_0}$ is the initial particle radius, $\bar{a}$ is the actual particle radius, K is the coalescence constant and t is the time.

The coarsening of emulsions through molecular diffusion is due to the gradual growth of the larger droplets of the emulsion at the expense of smaller ones. This mechanism was proposed by Lord Kelvin based on the fact that individual molecules tend to leave the smaller particles and diffuse through the continuous phase to join the larger ones [Thomson (1871)] Thus, the particle growth is achieved without physical contact of the smaller particles. According to the Theory of Lifshits and Slyozov (1961), the molecular diffusion mechanism can be characterized by a linearly micelle size growth with time

$$\frac{d\left(\bar{a}^3\right)}{dt} = \frac{8CD\gamma V}{9RT} \qquad (2)$$

where $\bar{a}$ is the particle radius, t is the time, C and D are, respectively, the solubility and the diffusion coefficient of the dispersed phase in the continuous medium, V is the molar volume of the dispersed substance, γ is the interfacial tension between the dispersed and continuous phases, R is the molar gas constant, and T is the absolute temperature. By this mechanism the increase in volume of the micelles is proportional to the solubility and the diffusion coefficient of the dispersed phase (perfluorocarbon) in the continuous phase (water). Therefore, emulsions that undergo Ostwald ripening can be stabilized by decreasing the solubility of the dispersed phase in the continuous phase and/or by decreasing the interfacial tension between the two phases. Also, the molecular diffusion theory assumes that the coarsening rate of a particle is independent of its surrounding, that is, a micelle surrounded by micelles larger than itself will coarsen at the same rate of a micelle surrounded by smaller ones [Voorhees (1985)].

It has been suggested that molecular diffusion plays a decisive role in the coarsening of fluorocarbon-in-water emulsions (up to 50% (w/v)) and for particles less than 1 μm in diameter (Postel et al., 1994). Since the effective transport of adequate amounts of oxygen requires the development of considerably more concentrated injectable emulsions, it is necessary to verify whether or not molecular diffusion is still the primary degradation process and how the surfactant used may affect the ageing mechanism. The purpose of this study is to identify and understand the ageing mechanism for perfluorohexane (50 % (w/v)) emulsions and its dependence on the surfactant used.

MATERIAL AND METHODS

The fluorocarbon selected to perform this study was perfluorohexane (C_6F_{14}), from Sigma-Aldrich, 99% pure. Three surfactants were tested: lecithin (L-α-

Phosphatidylcholine) from egg yolk 60% (TLC) from Fluka, Pluronic F-68, 10% aqueous solution, and Span 20 both from Sigma-Aldrich. The water used in the emulsion preparation was double distilled water. Fluororocarbons and emulsifiers were used without any further purification.

Emulsions of 50%(w/v) of perfluorohexane in water using 5%(w/v) using each of the three surfactants were prepared by sonication, using a IKA Labortechnik sonicator, model U200Scontrol. The sonication was performed for 2 min, at cycle1, with a constant amplitude 80%. The emulsions stability was studied through the evolution of the mean particle size at room temperature, 298 K. The size of the micelles was measured using an optic microscope, Zeiss, Axioscop with a camera Zeiss, Axiocam HRC.

The oxygen solubility was measured using a Glucose (GO) Assay Kit from Sigma.

STABILITY EXPERIMENTAL RESULTS

The micelle diameter, for each emulsion, was measured periodically at six different times: immediately after preparation and after 42, 56, 93, 154 and 190 days. The microscopic images of the final state of the emulsions are presented in Figure 1. The evolution of the mean micelle diameter with time for each emulsion is presented in Table 1. The associated uncertainty in the mean micelle diameter was calculated for a universe of 60 objects in a 95% confidence interval (Miller and Miller, 1993). The larger uncertainties found for the larger micelles can be explained by the fact that as emulsions loose their stability not only the mean micelle diameter increases but also the particle size distribution suffers a broadening leading to higher standard deviations.

The data on Table 1 clearly shows all the emulsions have micelles smaller than 1 μm, the one prepared with Pluronic F-68 exhibits the smaller average micelle diameter, even

at the final stage. The less stable emulsion for long periods of time, is the one prepared with Span 20, which displays the larger average micelle diameter after 190 days. Note however, that for shorter times, up to 50 days, this emulsion is the most stable, and that, up to 100 days, all the three emulsions have very similar average micelle diameters.

To verify the absence of systematic errors, a statistical analysis of the experimental data was performed. In Figure 2, the Gaussian distributions of the micelles sizes obtained for each emulsion at different times are presented, showing that there are no significant systematic errors (or bias) Also, the fact that the mean diameter obtained from these micelles size distributions and the experimental diameter are very close, shows that the experimental results obtained are precise. Note also that this behavior is time invariant. The only emulsion that deviates from a Gaussian behavior is the emulsion with Pluronic F-68 for times immediately after preparation. This can be explained by the impossibility to detect micelles smaller than 0,5 μm that are in the resolution limit of the microscopic technique used.

The evolution of the micelle volume with time is presented in Figure 3. The volume of the micelles in the emulsions where lecithin and Span 20 were used, increases exponentially with time, thus following the theory of Van den Tempel, indicating that coalescence is the major process of stability loss for such perfluorohexane emulsions. This is confirmed by the fact that after 190 days these emulsions exhibit larger particle sizes than the Pluronic F-68 emulsion.

The Pluronic emulsion follows a different trend where the volume of the micelles increases linearly with time, indicating that the primary mechanism that governs its ageing is Ostwald ripening. It can also be observed in Figure 3 that this emulsion presents the smaller particles (≈1 μm) at the final state. Interestingly for shorter times,

the low coalescence constant for Span 20 leads to more stable emulsions than Pluronic F-68.

It should be noted that the regression coefficients for the fittings depicted in Figure 3 are around 0.9, indicating an acceptable description of the data with the established theories. The results obtained indicate that the ageing of the concentrated water-in-perfluorocarbon emulsions depends on the surfactant used and, unlike for diluted emulsions (Postel et al., 1994), the Ostwald Ripening mechanism is not responsible for the ageing of all emulsions. The very low coalescence constant that some surfactants, such as Span 20, show can also mislead the conclusions about the ageing mechanism unless very long ageing times are considered.

Partial sedimentation was observed after some time for the three emulsions. Only the Pluronic F-68 emulsion could be re-homogenised by manual shaking, indicating that micelle coalescence had not taken place for this emulsion [Varescon et al. (1989)].

OXYGEN SOLUBILITY RESULTS

A new precise and expedite enzymatic method for measuring the oxygen content in perfluorocarbon emulsions is proposed. This method is based in the oxidation of glucose by molecular oxygen catalyzed by glucose oxidase, and is commonly used for dosing glucose when oxygen in excess is present [Sloviter et. al (1970)]. The method was adapted to measure the molecular oxygen when glucose is present in excess at 310.15 K.

The linear relationship between the amount of glucose oxidized and the amount of the emulsion used indicates the validity of this method to measure the dissolved oxygen in dispersed perfluorocarbons. The molar ratio of glucose to oxygen in this experiment was found to be 1.2.

The experimental solubility results obtained are listed in Table 2. They clearly show that the oxygen solubility is independent of the surfactant used, since they are of the same order of magnitude. Also, comparison with literature results for the solubility of oxygen in perfluoro-n-hexane [Costa Gomes et. al. (2003)], at the same temperature indicates that the solubility in the emulsion is lower than in the perfluorocarbon alone. Similar results have been reported for other perfluorocarbon emulsions using different methods to evaluate the oxygen dissolved [Sharts and Reese (1978)]

CONCLUSIONS

In this work the stability of perfluoro-n-hexane 50 % (w/v) emulsions using three different surfactants, lecithin, Span 20 and Pluronic F-68, was studied. The results indicate that the ageing mechanism depends on the surfactant used: the emulsions prepared with lecithin and Span 20 loose stability by coalescence while Ostwald ripening is the primary coarsening mechanism of the Pluronic F-68 emulsion. This conclusion can only be drawn in a long term study, 190 days. Note that if only data up to 100 days was considered the conclusions would be different.

The oxygen solubility in the perfluorocarbon emulsions was also studied using an enzymatic method. The results obtained show that the oxygen solubility in the three emulsions are of the same order of magnitude, thus indicating that the surfactant does not play a dominant role in this property.

ACNOWLEDGMENTS

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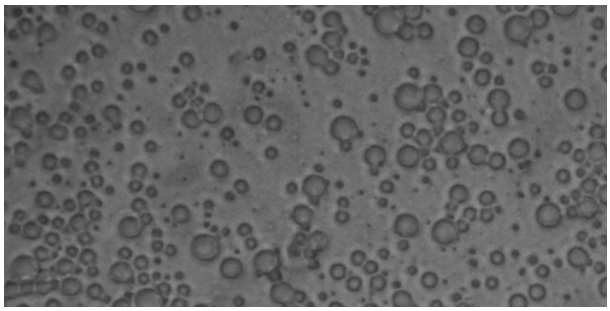
ILLUSTRATIONS AND TABLES



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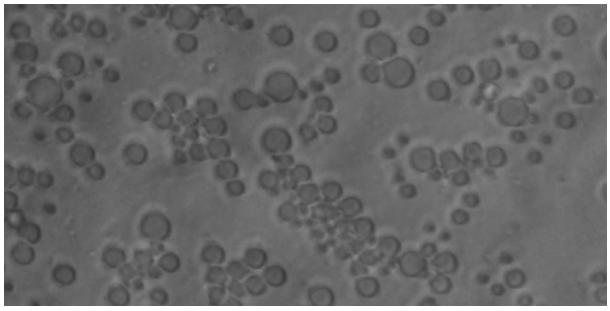

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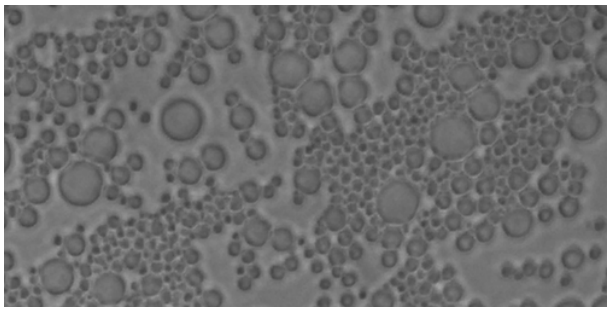
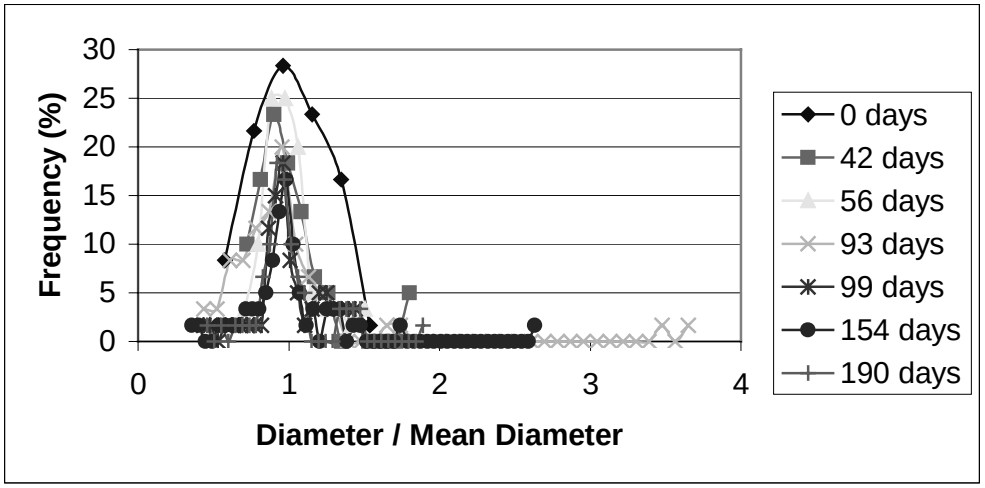
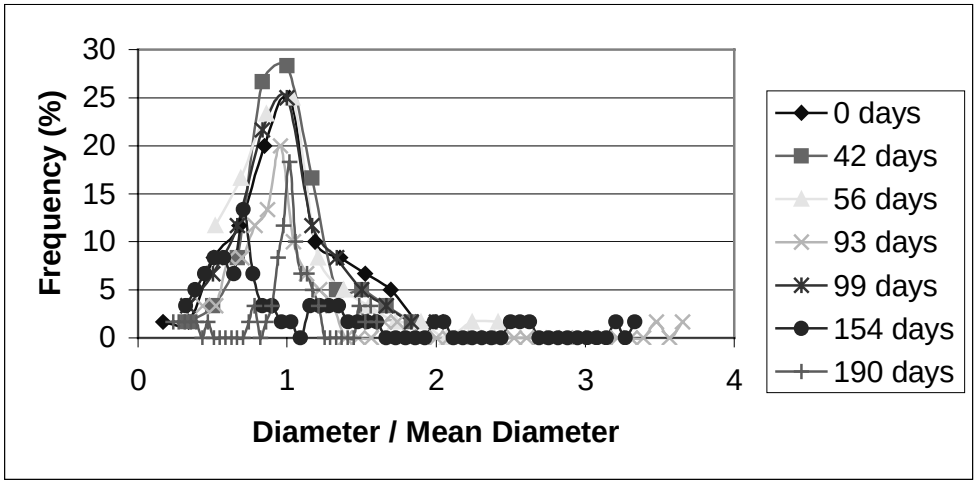


(C)

Figure 1 – Microscopic images for the final state of the for Lecithin Emulsion (A), Span Emulsion (B) and Pluronic Emulsion (C) (Magnification: 400x)



(A)



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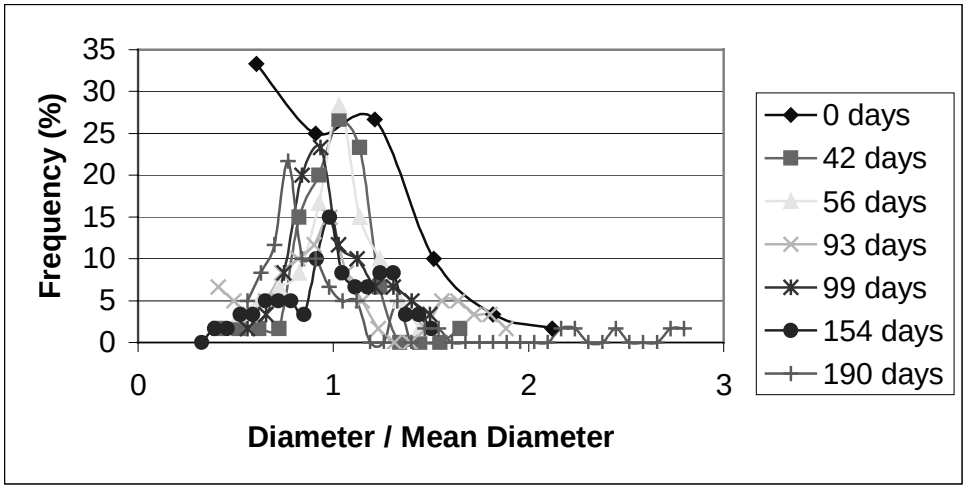

(C)

Figure 2 – Variation of the distribution function of micelle diameter as function of time for Lecithin Emulsion **(A)**, Span Emulsion **(B)** and Pluronic Emulsion **(C)**

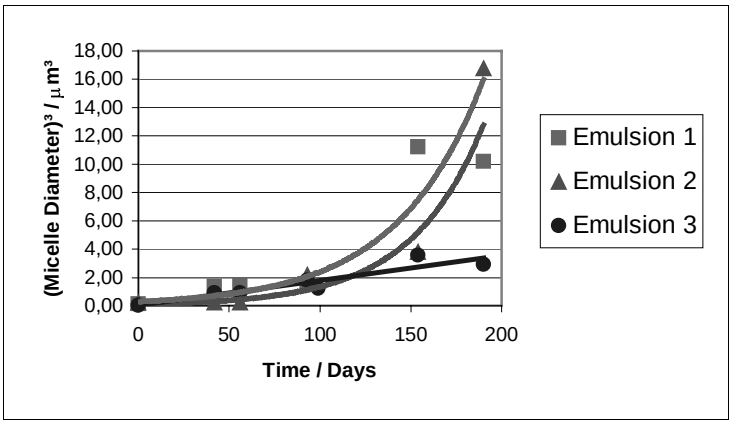

Figure 3 – Cube of the micelle mean diameter as function of time, at room temperature for the Emulsion 1 (50% (w/v) C_6F_{14} + 5% (w/v) Lecithin), Emulsion 2 (50% (w/v) C_6F_{14} + 5% (w/v) Span 20), Emulsion 3 (50% (w/v) C_6F_{14} + 5% (w/v) Pluronic F-68),

Table 1 – Evolution of the mean particle size with time in Emulsion 1 (50% (w/v) C_6F_{14} + 5% (w/v) Lecithin), Emulsion 2 (50% (w/v) C_6F_{14} + 5% (w/v) Span 20), Emulsion 3 (50% (w/v) C_6F_{14} + 5% (w/v) Pluronic F-68).

	Micelle Diameter / (μm)		
Time / (days)	**Emulsion 1**	**Emulsion 2**	**Emulsion 3**
0	0.52 ± 0.03	0.59 ± 0.05	0.33 ± 0.03
42	1.11 ± 0.07	0.60 ± 0.04	0.97 ± 0.05
56	1.13 ± 0.05	0.58 ± 0.06	0.97 ± 0.05
93	1.2 ± 0.2	1.3 ± 0.2	1.22 ± 0.12
154	2.2 ± 0.2	1.0 ± 0.3	1.53 ± 0.10
190	2.2 ± 0.1	2.56 ± 0.19	1.43 ± 0.19

Table 2 – Concentration of oxygen in the studied perfluorocarbon emulsions.

Surfactant	O2 content of emulsion, ml / 100 ml
Lecithin	3.11
Span 20	2.59
Pluronic F-68	2.70